The Journal of Veterinary Medical Science

FULL PAPER

Surgery

Renal ultrasonographic strain elastography and symmetric dimethylarginine (SDMA) in canine and feline chronic kidney disease

Chutimon THANABOONNIPAT[1,2], Saikaew SUTAYATRAM[1], Chollada BURANAKARL[1] and Nan CHOISUNIRACHON[2]*

[1]Department of Veterinary Physiology, Faculty of Veterinary Science, Chulalongkorn University, 39 Henri-Dunant Road, Wangmai, Pathumwan, Bangkok, Thailand

[2]Department of Veterinary Surgery, Faculty of Veterinary Science, Chulalongkorn University, 39 Henri-Dunant Road, Wangmai, Pathumwan, Bangkok, Thailand

J. Vet. Med. Sci.
82(8): 1104–1112, 2020
doi: 10.1292/jvms.19-0496

Received: 5 September 2019
Accepted: 6 June 2020
Advanced Epub:
18 June 2020

ABSTRACT. Chronic kidney disease (CKD) is a common renal disease in dogs and cats. Renal fibrosis is a main pathologic process leading of CKD progression. Renal biopsy is the gold standard for renal fibrosis assessment. However, it is not routinely performed in clinic due to its invasiveness. Therefore, the aim of this study was to evaluate the use of ultrasonographic strain elastography (SE), which is a non-invasive method for renal tissue stiffness determination and its association with renal function. Renal strain ratios and renal function were evaluated in 13 CKD dogs (CKDD), 38 healthy dogs (HD), 17 CKD cats (CKDC) and 26 healthy cats (HC). There were significantly lower renal cortical strain ratios than medullary strain ratios in all groups (HD; $P<0.01$, HC; $P<0.01$, CKDD and CKDC; $P<0.05$) and significantly lower cortical and medullary strain ratios in both CKDD and CKDC than in healthy control animals of both species ($P<0.0001$). In dogs, the renal cortical and medullary strain ratios significantly negatively correlated with plasma creatinine ($P<0.05$), blood urea nitrogen (BUN; $P<0.05$; $P<0.01$, respectively), and symmetric dimethylarginine (SDMA; $P<0.01$). In cats, similar correlations were found for plasma creatinine ($P<0.001$), BUN ($P<0.05$; $P<0.001$, respectively) and SDMA ($P<0.05$). SE might be a promising imaging diagnostic tool for renal-elasticity evaluation, also correlating with renal functional impairment in canine and feline CKD.

KEY WORDS: cat, chronic kidney disease (CKD), dog, strain elastography, symmetric dimethylarginine (SDMA)

Chronic kidney disease (CKD) is an irreversible and typically progressive disease with a high prevalence in both dogs and cats [26]. CKD is defined by the presence of structural and/or functional abnormalities existing for a long period of time, normally 3 months or longer [22, 26].

In clinical practice, blood creatinine (Cr) evaluation is a standard and acceptable renal function test routinely performed together with blood urea nitrogen (BUN) measurement. The International Renal Interest Society (IRIS) has proposed a progressing 4-stage scoring system for CKD in both dogs and cats classified based on Cr concentrations, degree of proteinuria and blood pressure in order to facilitate diagnosis, treatment, prognosis, and research [8, 17]. Recently, the IRIS released a new guideline for CKD staging in dogs and cats that introducing a new renal biomarker, symmetric dimethylarginine (SDMA), to the IRIS staging system [17].

SDMA can represent the glomerular filtration rate (GFR) [5, 13, 14] as it originates from post-translational methylation of arginine residues in proteins of any nucleated cell [13, 14] and is eliminated almost completely by renal excretion [5–7]. Compared with Cr, blood SDMA has higher sensitivity for detecting renal function. SDMA increases with only 25 to 40% reduction in GFR, whereas increase in Cr cannot be detected until GFR is reduced by 75% [13, 14]. Moreover, SDMA is not influenced by extra-renal factors such as muscle mass, which is an important limitation of Cr [13]. Thus, blood SDMA can assist the veterinarian in early CKD diagnosis and staging especially in patients with cachexia. Therefore, early diagnosis and management of CKD could be improved leading to slower disease progression [14].

In addition to SDMA measurements, ultrasonographic elastography is a new diagnostic imaging technique that can assess tissue elasticity or tissue stiffness [23]. Strain elastography (SE) is an ultrasonographic elastography technique that measures

*Correspondence to: Choisunirachon, N.: nan.c@chula.ac.th

tissue elasticity after force application from manual ultrasound-transducer compression on tissue or force exertion from internal physiological movement such as the heartbeat and respiration [2]. A semi-quantitative measure termed the strain ratio can be used to compare between tissues of interest [29]. In humans, it has been reported that patients with CKD have significantly lower strain values compared to healthy individuals [20]. Therefore, SE can be effectively used for noninvasive detection and monitoring of renal elasticity in patients with CKD, which might represent the degree of renal fibrosis [1].

Presently, information regarding renal SE in veterinary medicine is only available for dogs [18] and cats [33] with normal kidney function. Additionally, no study has reported on the relationship between the renal elasticity observed though the strain ratio from SE and SDMA. Therefore, the purposes of this study were, first, to determine the relationships between the renal strain ratio and age, sex, and body weight in both dogs and cats. Second, to compare the renal strain ratios in healthy and CKD animals of both species. Third, to investigate the relationships between the renal strain ratios and renal function parameters including plasma concentrations of Cr, BUN, and SDMA in both dogs and cats.

MATERIALS AND METHODS

The present study protocol was approved by the Chulalongkorn University Animal Care and Use Committee (CU-ACUC), Faculty of Veterinary Science, Chulalongkorn University (Protocol number: 1731055). All dogs and cats that were presented to the Small Animal Teaching Hospital, Faculty of Veterinary Science, Chulalongkorn University from September to December 2017 were considered for inclusion. The client-owned dogs and cats were divided into healthy dog (HD; n=38), CKD dog (CKDD; n=13), healthy cat (HC; n=26) and CKD cat (CKDC; n=17) groups. The owners were informed and provided signed consent before the procedures. All the enrolled dogs and cats were included only once in the present study and all procedures were done within a visit day. The HD and HC groups consisted of healthy volunteer dogs and cats that presented normal results of history, physical examination, routine blood examination including complete blood count (CBC), plasma biochemistry, microscopic examination of blood parasites, abdominal radiography, and B-mode abdominal ultrasonography. All cats included in the HC group had negative results for feline immunodeficiency virus (FIV) and feline leukemia virus (FeLV) infection using ELISA test kits (WITNESS® FeLV-FIV; Zoetis, Madison, NJ, USA). Conversely, the CKDDs and CKDCs included in this study were selected based on a history of either structural or functional renal abnormalities with Cr equal to or higher than 1.4 and 1.6 mg/d*l* for dogs and cats, respectively, for more than 3 months and were categorized into the IRIS stages according to the IRIS staging system [17]. Both CKDDs and CKDCs were subjected to the same diagnostic procedures as the HD and HC groups. CKDCs also had negative results for FIV and FeLV infection based on ELISA testing (WITNESS® FeLV-FIV; Zoetis). In addition, dogs and cats that presented with any infectious diseases, lymphoma, congenital kidney diseases such as polycystic kidney, and hydronephrosis were excluded from this study.

Blood samples from dogs and cats were collected in ethylenediaminetetraacetic acid (EDTA) tube (K3 EDTA collection test tube; FL MEDICAL, Torreglia, Italy) for CBC measurement on the same visiting day and lithium heparin tubes (VACUETTE®; Greiner Bio-One GMbH, Frickenhausen, Germany) for plasma preparation. Subsequently, the plasma samples were stored at −20°C before determining the concentrations of Cr, BUN, alanine aminotransferase (ALT), alkaline phosphatase (ALP), total protein, albumin and SDMA. Urine samples from dogs and cats were also collected to measure the urine protein to creatinine ratio (UPC). Microscopic observation of the urine sediment was performed before UPC measurement in order to exclude the urinary tract infection. The UPC ratio was examined by measuring the urine protein concentrations after precipitation with 3% sulphosalicylic acid [32]. The blood pressure measurement was performed using a Doppler blood pressure instrument (Vet-Dop 2TM; Vmed Technology, Mill Creek, WA, USA).

Plasma Cr concentrations, BUN, ALT, ALP, total protein and albumin were analyzed using an automated analyzer (The IL ILab 650 Chemistry Analyzer; Diamond Diagnostics, Holliston, MA, USA). Plasma concentrations of SDMA were analyzed using an automated analyzer (Catalyst One Chemistry Analyzer; IDEXX Laboratories, Westbrook, ME, USA) and the Catalyst SDMA test (IDEXX SDMA test; IDEXX Laboratories). All procedures were performed following the manuals' guidelines.

Abdominal radiographic and ultrasonographic examinations were performed without sedation or anesthesia. For abdominal ultrasonography, all dogs and cats were manually restrained and positioned on either the right or left lateral recumbency for each ipsilateral site of the renal observations. Preparation for ultrasonographic examinations by hair clipping and application of acoustic coupling gel to the skin was performed. All dogs and cats were screened over the whole abdominal organs, especially the kidneys, in various planes, i.e., the sagittal, dorsal, and transverse planes with B-mode abdominal ultrasonography using a 9 MHz-bandwidth linear transducer (DC-8 EXP; Mindray Medical International, Shenzhen, China). SE was then performed on the sagittal plane of each kidney by manual compression with the ultrasound transducer. Elastography images were displayed on a dual screen including a B-mode image and an SE color qualitative elastogram overlaid on B-mode (Figs. 1 and 2). The elastography images were collected with the transducer in perpendicular orientation to the kidney and abdominal wall. The variations of tissue strain caused by manual compression are displayed as the strain graph showing the averaged strain value at different time points in the region of interest (ROI). The operator inspected the stability and uniformity of compression by monitoring the strain graph in real time and adjusted the compression before selecting only the stable strain graphs of regular shape (Figs. 1 and 2). The elastography color scale uses red color to represent the hardest tissues and blue color to represent the softest tissues. The fixed ROIs were manually drawn as wide as possible to cover the selected cortex and medulla of the kidney together with the adjacent superficial and near-field abdominal wall. Three separate elastography images were selected. In each elastography image, the kidney strain ratio was measured as the ratio of the strain value of the renal parenchyma (renal cortex or renal medulla) to that of the near-field

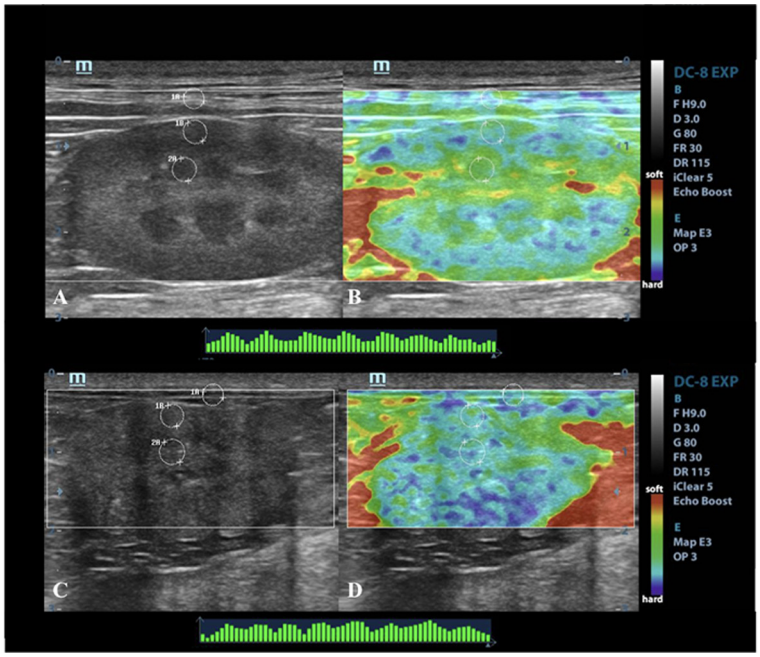

Fig. 1. Renal strain elastography images between a healthy dog (HD). A, B comparing to a chronic kidney disease dogs (CKDD); C, D. A and C were B-mode ultrasound images. B and D were color elastograms overlaid on the ROI on the B-mode. Regions of interest (ROIs) were circularly drawn over the mid renal cortex, medulla and superficial abdominal wall to calculate the renal strain ratios.

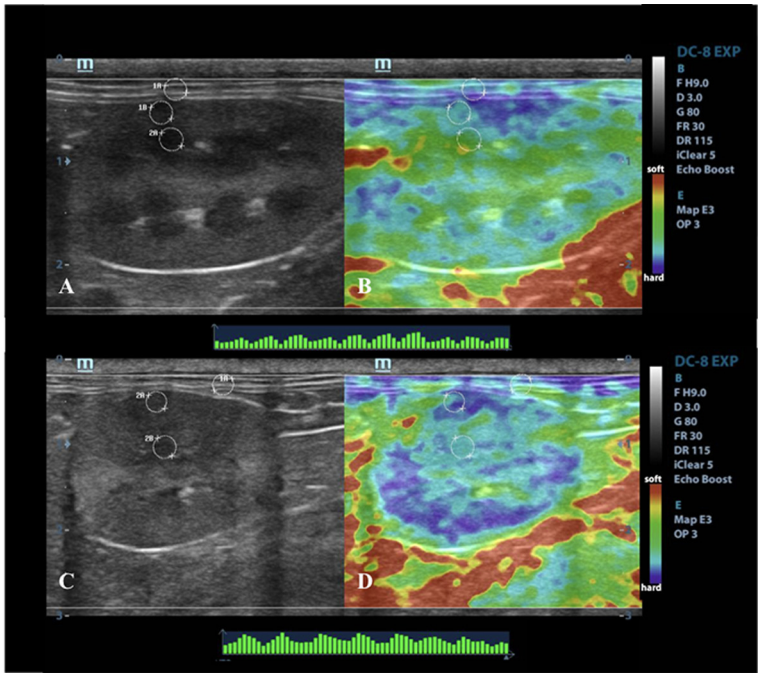

Fig. 2. Renal strain elastography images between a healthy cat (HC). A, B comparing to a chronic kidney disease cats (CKDC); C, D. A and C were B-mode ultrasound images. B and D were color elastograms overlaid on the ROI on the B-mode. Regions of interest (ROIs) were circularly drawn over the mid renal cortex, medulla and superficial abdominal wall to calculate the renal strain ratios.

abdominal wall within the same image and calculated as described in a previous study in which: strain ratio=mean strain value of organ parenchyma/ mean strain value of the abdominal wall [18]. The strain ratios from three separate elastography images were then averaged.

All analyses were performed using SAS (SAS version 9.0; Cary, NC, USA). Results are expressed as mean ± standard deviations (SD). Normality distributions were tested with the Shapiro-Wilk test. The data among groups were compared using multiple analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS. The dependent variables in the statistical models included groups (Healthy; HD or HC and Chronic kidney disease; CKDD or CKDC), sides (left and right), layers (cortex and medulla) and the two-way interactions. Least square means were obtained from each class and were compared by the least significant difference test using Bonferroni adjustment for multiple comparison. Spearman's correlation coefficient was applied to investigate the correlations between parameters and polynomial regression was selected based on the Akaike's criterion (AIC) values. The estimated sensitivity, specificity and area under the curve (AUC) were evaluated by receiver operating characteristics (ROC) curve analysis. For all statistical tests, $P<0.05$ was considered to be statistically significant.

RESULTS

In total, 38 HD and 13 CKDD, and 26 HC and 17 CKDC were included in this study. The demographic data of all dogs and cats groups are presented in Table 1. The mean ages of HD and CKDD in this study were comparable. In contrast, the mean age of CKDC was higher than that of HC. The HD were Shih Tzu (20), Yorkshire Terrier (6), Golden Retriever (3), Cocker Spaniel (2), Chihuahua (1), Pomeranian (1), Siberian Husky (1), Cardigan Welsh Corgi (1), Poodle (1), Maltese (1), and Pekingese (1), the CKDD were Shih Tzu (5), mixed breed (3), Chihuahua (2), Domestic Thai (1), Siberian Husky (1) and American Pitbull Terrier (n=1). The HC were Domestic shorthair (18), Persian (6), and American Shorthair (2), and the CKDC were Domestic Shorthair (16) and Persian (1). The CKDD were classified into IRIS stage 2 (53.3%) and stage 3 (46.7%), while the CKDC were classified to IRIS stage 2 (82.6%), stage 3 (13.1%) and stage 4 (4.3%).

On standard B-mode ultrasonography, the kidneys of the HD and HC groups were normal in shape, margin, contour, echogenicity, and echotexture, whereas the renal B-mode ultrasonographic findings of the CKDD and CKDC groups were increase in both cortical and/or medullary echogenicity, loss of corticomedullary demarcation zone, and irregular contour.

The functional renal parameters including plasma concentrations of Cr, BUN and SDMA of all dog and cat groups are summarized in Table 2. The plasma Cr, BUN and SDMA concentrations of CKDD were significantly higher than those of HD ($P<0.001$). Similarly, the CKDC also had significantly higher concentrations of plasma Cr, BUN and SDMA than those of HC (Cr and BUN; $P<0.001$, SDMA; $P<0.01$). The UPC ratio of the CKDD group was significantly higher than that of the HD group (0.04

Table 1. Geographic information of healthy dogs (HD), chronic kidney disease dogs (CKDD) groups and healthy cats (HC) and chronic kidney disease cats (CKDC) groups

Parameters	HD (n=38)	CKDD (n=13)	HC (n=26)	CKDC (n=17)
Sex				
Female	24	4	13	8
Intact	20	2	6	4
Neutered	4	2	7	4
Male	14	9	13	9
Intact	11	6	6	4
Neutered	3	3	7	5
Age (years)	8.3 ± 3.9 (0.7–15.0)	11 ± 5.4 (2–21)	4.4 ± 3.4 (0.7–10)	10.1 ± 5.0 (3.0–21.0)
Body weight (kg)	8.0 ± 8.1 (1.7–35)	11.5 ± 11.7 (1.2–39.4)	4.1 ± 0.9 (2.9–5.9)	3.7 ± 0.9 (2.6–5.9)
Body condition score	3.3 ± 0.5 (2.5–4)	3.0 ± 0.6 (2–4)	3.1 ± 0.6 (2.0–4.0)	3.0 ± 0.4 (2.0–4.0)

Data are presented as mean ± SD and range.

Table 2. The renal functional parameters including plasma concentrations of creatinine, blood urea nitrogen (BUN) and symmetric dimethylarginine (SDMA) of the healthy dogs (HD), chronic kidney disease dogs (CKDD), the healthy cats (HC) and chronic kidney disease cats (CKDC)

Parameters	HD	CKDD	HC	CKDC
Creatinine (mg/d*l*)	0.8 (0.5–1.3)	2.2 (1.4–4.0)[b)]	1.3 (0.9–1.6)	2.3 (1.6–5.3)[b)]
BUN (mg/d*l*)	18.6 (9.3–21.0)	42.7 (17.2–83.3)[b)]	21.0 (14.0–31.9)	31.6 (17.6–109.5)[b)]
SDMA (μg/d*l*)	13.5 (9.0–17.0)	37.0 (18.0–87.0)[b)]	15.0 (12.0–18.0)	22.0 (15.0–53.0)[a)]

Normal reference intervals considered for dogs were as follows: Creatinine=0.3–1.4 mg/d*l*; BUN=5–21 mg/d*l*; SDMA=0–18 μg/d*l*. Normal reference intervals considered for cats were as follows: Creatinine=0.6–1.6 mg/d*l*; BUN=14–36 mg/d*l*; SDMA=0–18 mg/d*l*. BUN blood urea nitrogen; SDMA, symmetric dimethylarginine. Data are presented as median and range. Statistically differences between HD and CKDD, and HC and CKDC groups were made using Unpaired *t*-test, a) $P<0.01$; b) $P<0.001$.

Table 3. Strain values of renal parenchyma, the near-field abdominal wall and renal strain ratios in healthy dogs (HD) and chronic kidney disease dogs (CKDD)

	Left kidney		Right kidney	
	Cortex	Medulla	Cortex	Medulla
HD				
Parenchyma	0.45 ± 0.16	0.49 ± 0.11	0.45 ± 0.09	0.49 ± 0.12
Abdominal wall	0.22 ± 0.04	0.22 ± 0.04	0.22 ± 0.06	0.22 ± 0.06
Strain ratio	2.10 ± 0.45	2.32 ± 0.54	2.12 ± 0.62	2.33 ± 0.69
CKDD				
Parenchyma	0.30 ± 0.09	0.34 ± 0.06	0.32 ± 0.06	0.37 ± 0.05
Abdominal wall	0.22 ± 0.05	0.22 ± 0.05	0.24 ± 0.08	0.24 ± 0.08
Strain ratio	1.42 ± 0.50	1.57 ± 0.40	1.48 ± 0.56	1.67 ± 0.50

Data are presented as mean ± SD.

Table 4. Strain values of renal parenchyma, the near-field abdominal wall and renal strain ratios in healthy cats (HC) and chronic kidney disease cats (CKDC)

	Left kidney		Right kidney	
	Cortex	Medulla	Cortex	Medulla
HC				
Parenchyma	0.44 ± 0.11	0.50 ± 0.12	0.38 ± 0.11	0.47 ± 0.12
Abdominal wall	0.22 ± 0.05	0.22 ± 0.05	0.19 ± 0.04	0.19 ± 0.04
Strain ratio	2.10 ± 0.53	2.33 ± 0.56	2.33 ± 0.66	2.52 ± 0.75
CKDC				
Parenchyma	0.32 ± 0.08	0.34 ± 0.08	0.30 ± 0.06	0.35 ± 0.09
Abdominal wall	0.28 ± 0.09	0.28 ± 0.09	0.29 ± 0.08	0.29 ± 0.08
Strain ratio	1.19 ± 0.31	1.29 ± 0.39	1.10 ± 0.35	1.29 ± 0.42

Data are presented as mean ± SD.

± 0.07 and 1.97 ± 3.21, respectively; $P<0.001$). In cats, the UPC ratio of the CKDC group was also significantly higher than that of the HC group (0.09 ± 0.1 and 0.97 ± 1.72, respectively; $P<0.001$).

The average blood pressure of the HD group was significantly lower than that of the CKDD group (120 ± 22 mmHg and 141 ± 22 mmHg, respectively; $P<0.05$). Similarly, in cats, the average blood pressure of the HC group was also significantly lower than that of the CKDC group (129 ± 17 mmHg and 151 ± 26 mmHg, respectively; $P<0.01$).

For the SE procedure, in the HD and HC groups, the corticomedullary demarcation and border between the renal margins and surrounding perirenal tissues were distinctly visible, and the operator could apply sufficient strain through the transducer over the renal cortex and medulla in both kidneys, especially in the HC group. However, when comparing the renal SE success rates between the right and left kidneys in the HD and HC groups, the results showed that HD had a lower right renal SE success rate than left renal SE success rate (90% and 100%, respectively). In contrast, the HC group could be completely evaluated for both right and left renal SE in all cats (100%). For the CKDD and CKDC, the results showed that CKDD also had a lower right renal SE success rate than left renal SE success rate (66.7 and 93.3%, respectively), whereas renal SE could be successfully performed in all CKDC in both the right and left kidneys (100%).

The mean strain values for each kidney and abdominal wall and the renal strain ratios observed with the SE in HD and CKDD and HC and CKDC are shown in Tables 3 and 4, respectively. The average renal strain ratios of the renal cortex and medulla were not significantly different between the right and left kidneys in each group (Tables 3 and 4). Age, bodyweight, and body condition score (BCS) did not affect the average renal strain ratios of the cortex or medulla in HC. In HD, age, bodyweight, and the BCS also did not affect the average renal cortical strain ratios. However, bodyweight and the BCS had a significant positive correlation with the average renal medulla strain ratios in the HD group (bodyweight; $P<0.05$, r=0.294 and BCS; $P<0.05$, r=0.321). Considering the effect of sex, the average renal strain ratios of male and female dogs and cats in the within group comparisons were non significantly different.

Regarding to the average renal cortex and medulla strain ratios, the results indicated that the average renal strain ratios of the renal cortex in all groups were significantly lower than those of the renal medulla (HD; $P<0.01$, HC; $P<0.01$, CKDD; $P<0.05$ and CKDC; $P<0.05$). Moreover, the average renal cortical and medullary strain ratios of CKDD were significantly lower than those of HD ($P<0.0001$) (Fig. 3A). These findings were also detected in cats, where CKDC had the average renal cortical and medullary strain ratios significantly lower than those of HC ($P<0.0001$) (Fig. 3B).

The correlations between the average renal strain ratios and plasma concentrations of Cr, BUN or SDMA in the 51 dogs and 43 cats from both healthy and CKD animals of each species were evaluated. In dogs, both the renal cortical and medullary strain ratios

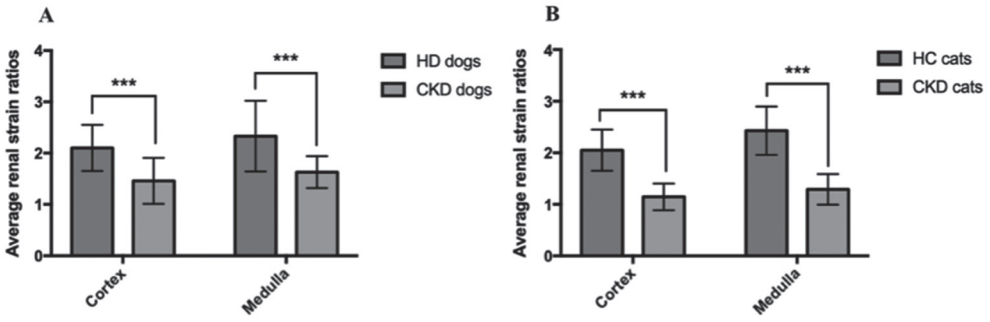

Fig. 3. Average renal strain ratios of cortex and medulla (mean ± SD) between the healthy dogs (HD) and chronic kidney disease dogs (CKDD), and the healthy cats (HC) and chronic kidney disease cats (CKDC). ***Statistically difference among groups were made using Bonferroni adjustment for multiple comparison, $P<0.0001$.

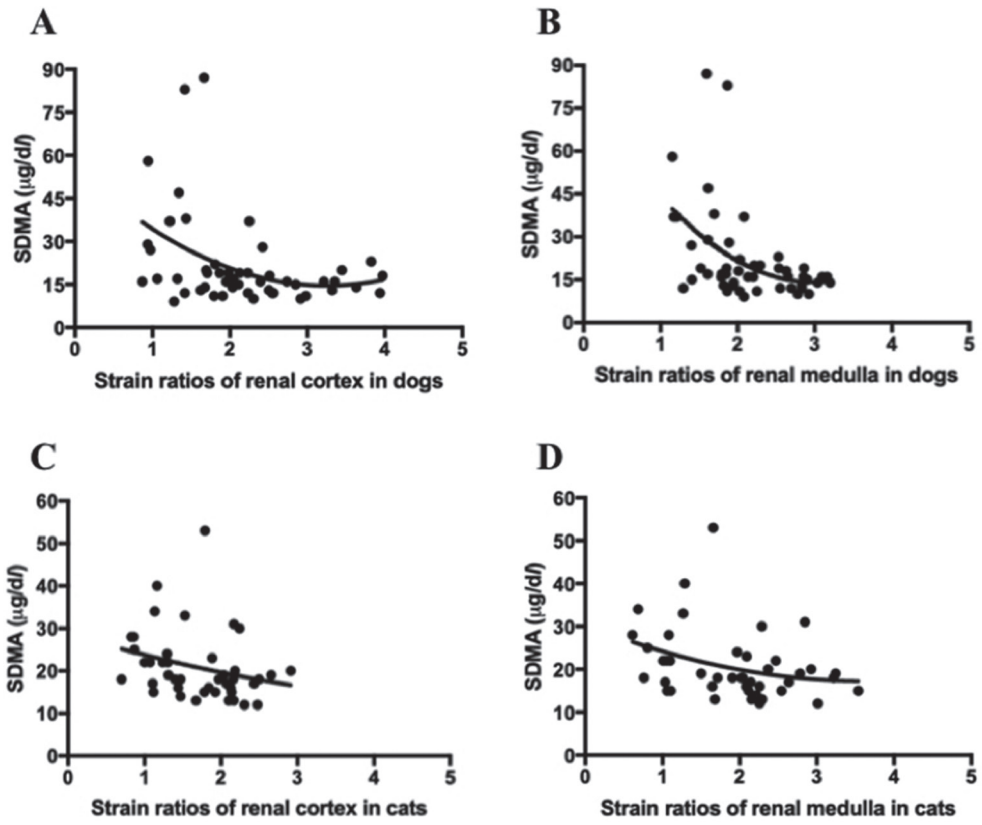

Fig. 4. Polynomial regression graph of either average renal cortical strain ratios or average renal medulla strain ratios and plasma symmetric dimethylarginine (SDMA) concentrations in dogs and cats using second order polynomial regression. A. Average renal cortical strain ratios and plasma SDMA in dogs. B. Average renal medulla strain ratios and plasma SDMA in dogs. C. Average renal cortical strain ratios and plasma SDMA in cats. D. Average renal medulla strain ratios and plasma SDMA in cats.

showed a significant negative correlation with concentrations of plasma Cr ($P<0.05$, r=–0.230; $P<0.05$, r=–0.250, respectively), BUN ($P<0.05$, r=–0.280; $P<0.01$, r=–0.370, respectively) and SDMA ($P<0.01$, r=–0.364 (Fig. 4A); $P<0.01$, r=–0.431 (Fig. 4B), respectively). In cats, both the renal cortical and medullary strain ratios also presented a significant negative correlation with concentrations of plasma Cr ($P<0.001$, r=–0.563; $P<0.001$, r=–0.674, respectively), BUN ($P<0.05$, r=–0.304; $P<0.001$, r=–0.496, respectively) and SDMA ($P<0.05$, r=–0.337 (Fig. 4C); $P<0.05$, r=–0.332 (Fig. 4D), respectively).

For the ROC curve and the AUC between the healthy and CKD groups of renal strain ratios, the AUC of the ROC curve between HD and CKDD was 0.908 with a 95% confidence interval 0.828 to 0.987 ($P<0.001$; Fig. 5A) and the AUC of the ROC curve between HC and CKDC was 0.991 with a 95% confidence interval 0.992 to 1.00 ($P<0.001$; Fig. 5B). The optimal cut-off value of ROC curve in dogs was lower than 1.72 of the strain ratio with 80% sensitivity and 89.19% specificity. The optimal cut-off value

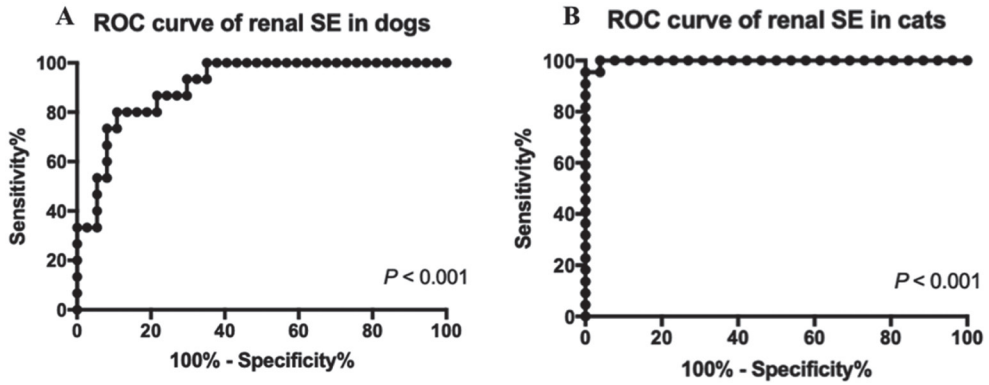

Fig. 5. Receiver Operating Characteristics (ROC) curve of the renal Strain elastography (SE) between healthy and chronic kidney disease (CKD) groups in dogs and cats using ROC curve analysis. A) ROC curve of the renal SE between healthy dogs (HD) and CKD dog (CKDD). B) ROC curve of the renal SE between healthy cat (HC) and CKD cat (CKDC).

of the ROC curve in cats was lower than 1.64 of strain ratio with 95.45% sensitivity and 96.15% specificity.

DISCUSSION

Ultrasonographic elastography is a non-invasive imaging technique used for tissue or parenchymal elasticity assessments by evaluating tissue capacity to deform when stressed and to return to the original shape after removing the stress [30]. According to these properties, this technique can differentiate normal tissue from pathological tissue with elasticity alteration such as increased stiffness, especially that resulting from a fibrotic process [4, 24].

SE is the first elastography technique that was developed in the 1990s [23] and is also one of the most popular ultrasonographic elastography techniques that measure tissue elasticity based on quasi-static tissue deformation after compression stress is applied using a probe [10]. Thus, the success of SE highly depends on operator experience [18, 33]. SE has continuously been developed by applying novel principles to create more advanced instruments that allow the ultrasound machine to qualitatively show tissue stiffness on a color image overlaid on the standard B-mode ultrasonography [9, 10]. In the present study, appropriate renal SE images were considered those showing uniform compression on the strain graph and mostly elastographic color uniformity [18, 21]. The renal parenchyma was compared to the superficial body wall in order to standardize the elasticity scale within each image [18, 21].

In veterinary medicine, a few studies have investigated SE utility on normal kidneys of healthy dogs [18] and healthy cats [33]. However, it has not been used in renal disease in both species. It has been reported that human patients with CKD have stiffer kidneys compared to healthy individuals, using SE and shear wave elastography (SWE); another elastographic technique for tissue stiffness determination [24]. The strain ratios from SE can be used in differentiating the CKD patients and healthy individuals in humans and can reflect the degrees of renal fibrosis in CKD patients [20]. However, this technique still cannot differentiate among stages of CKD in humans [20]. Recently, a SWE study in CKDC also affirmed that CKDC had lower kidney elasticity than those of HC [32]. Although, SWE has greater advantages than SE in which SWE can be used for real-time monitoring of both structural and tissue stiffness [2] and the reproducibility of SWE between observers is excellent [1]. However, the SWE instrument is more expensive and less available in clinical practice compared to the SE. As an alternative to SWE, SE may be also useful and applicable in the clinic for the investigation of renal parenchymal stiffness compared to conventional B-mode ultrasonography, especially in CKD dogs and cats.

For the SE procedure in companion animals, a good window could cover for renal SE in both kidneys, which correspond to the previous studies of SE in both dogs [18] and cats [33]. Conversely, this finding contradicts finding in humans that renal SE is limited due to the deeper location of the kidney [11]. However, considering the renal SE success rates for the right and left kidneys in both dogs and cats, our results indicated that the success rate of right renal SE was lower than that of left renal SE in the dogs in both the HD and CKDD groups. Moreover, the results also showed that the success rate of renal SE in dogs was lower than that in cats. Our findings correspond to previous results in dogs [18] where it was shown that achieving adequate compression force for right renal SE is anticipated to be more difficult than that required for left renal SE, as the right kidney is located more craniodorsally in some breeds, particularly in deep-chested dogs [25]. The renal SE success rate was also lower in dogs than in cats, possibly because of the location of the feline kidney, especially the right kidney that interferes less with the SE procedure compared to the right kidney in dogs, which is located more cranially and nearer to the rib cage [18, 28, 32]. As a result, respiratory motion could be one of the interfering factors when performing right kidney SE in dogs. To date, renal SE in humans has not been reported because of the kidney's deep location [11].

In agreement with the findings of previous studies in dogs [18] and cats [33], our results showed that the average renal strain ratios of the renal cortex and medulla were not significantly different between the right and left kidneys in both species. Moreover,

our findings also correspond to those of studies with dogs [15], cats [32] and humans [30] in which age, sex, bodyweight, and the BCS did not affect the average renal strain ratios or renal elasticity of the cortex or medulla in HC and the average renal cortical strain ratio or renal elasticity of the cortex in HD dogs. Interestingly, bodyweight and the BCS had a significant positive correlation with the average renal medulla strain ratios in the HD group. Considerable subcutaneous fat accumulation may affect the ability to apply sufficient compression stress to deform the deep parenchyma as the location of the renal medulla is deeper than that of the renal cortex. However, in this study, the small number of included dogs and cats is an important limitation in assessing the influence of demographic variables on elastography. Further studies should enroll more animals to elucidate the effect of the demographic variables.

The present study showed that the renal cortex had a lower strain ratio or renal elasticity than had the renal medulla in both the healthy and CKD dog and cat groups, which is consistent with previous study findings using SWE in cats [32], pigs [10] and humans [11, 24], and also corresponded to previous findings using magnetic resonance elastography in humans [3, 11, 31]. Conversely, contrasting results were reported by SE studies with healthy dogs [18] and cats [33]. The difference among studies might be due to differences in ultrasound instruments, picture acquisition techniques, and subject populations. Regarding our findings, the lower elasticity of the renal cortex compared with that of the renal medulla could be explained as previous described [32]. Renal parenchymal elasticity is highly influenced by the degree of vascular pressure, where a higher proportion of the cardiac blood flow is circulated to the renal cortex than to the renal medulla in the normal renal physiological condition [11].

Both the renal cortical and medullary SE strain ratios of CKDD were significantly lower than those of HD. This finding was also detected in cats in that CKDC had lower the renal strain ratios than those of HC. These results showed that CKD animals had increased kidney stiffness or decreased renal elasticity and correspond with those of studies with CKD cats [32] and humans [12, 16, 24, 27]. However, the results of this study showed that there was an overlap between the average renal strain ratios between the healthy and CKD dog and cat groups. This might limit the use of SE as a definitive diagnostic tool for CKD. Rather, SE should be applied as an adjunctive tool in order to provide earlier detection and more informative monitoring of renal elasticity in CKD in both species. As it is increasingly applied in humans, SE can identify the renal parenchymal elasticity in CKD in humans that have significantly lower elasticity compared to healthy individuals [20].

Additionally, our results revealed that the renal strain ratios of both the cortex and medulla in both dogs and cats had significant negative correlations with the concentrations of plasma Cr, BUN, and SDMA. These findings correspond to those of prior studies in cats [32] and humans [19] in that renal elasticity was associated with deterioration of renal function in patients with CKD. Moreover, from the ROC curve analysis, the AUC of renal SE between the healthy and CKD groups in both dogs and cats was good to excellent, indicating that this tool has high sensitivity for detecting renal elasticity deterioration, especially in patients with CKD. Our findings supported that renal SE might be a promising and adjunctive tool for canine and feline CKD evaluation and monitoring.

To our knowledge, information on renal SE in CKD dogs and cats has not been previously reported. In addition, the relationships between the renal elasticity observed through SE and renal functional parameters including plasma Cr, BUN and the newest renal function parameter, SDMA, have not been determined. This is the first study that reported on renal SE in CKD dogs and cats and the relationships of renal elasticity with renal function parameters. This information would be useful to clinical practitioners and researchers in future studies.

The major limitation of the present study was that we did not perform renal histopathological examination for comparison with the renal elasticity results from SE because the invasive biopsy procedure increases the risk of further renal damage especially in patients in poor condition such as in CKD animals. The second limitation is that the number of healthy dogs and cats included in the study was not sufficient to confirm the influence of demographic variables on SE. The third limitation is that most of the enrolled animals were in relatively earlier stages of CKD (IRIS stages 1 and 2). Further studies should include more animals with late stage CKD. The fourth limitation is that the causes of proteinuria could not be determined in this study. Another limitation is that we did not perform GFR measurement because the measurement method, such as the iohexol plasma clearance, requires a large volume of blood, which is challenging to obtain from animals in poor condition. Further study with a larger sample is required to provide more reliable information. Regarding the limitations of SE, the precision of the results remains highly dependent on operator skill. Moreover, this technique is more appropriate for the examination of superficial rather than of deeper organs, where it is more difficult to generate sufficient stress to the tissue of interest. However, this technique is still considered easy to use and is widely performed in the field of diagnostic imaging [18, 33].

In conclusion, SE can be used as an adjunctive diagnostic imaging modality for the evaluation and monitoring of CKD in both dogs and cats. There was lower elasticity in the renal cortex than in the renal medulla in all groups in this study, and the kidneys of CKD dogs and cats were stiffer than those of HD and HC, respectively. Moreover, the renal strain ratios also had negative correlations with plasma concentrations of Cr, BUN, and SDMA. Therefore, the results suggest that renal SE might be a promising and simple imaging diagnostic tool, which may be used in clinical practice as an implement for the detection and monitoring of CKD in dogs and cats.

ACKNOWLEDGMENTS. This study was granted from the 100th Anniversary Chulalongkorn University for Doctoral Scholarship, the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund) and the Overseas Research Experience Scholarship for Graduate Student from graduate school, Chulalongkorn University and Faculty of Veterinary Science, Chulalongkorn University. We would like to thank all technical recommendations from Mindray Medical (Thailand) and IDEXX (Thailand).

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